affinity corresponding to $K'_{diss} = 3.2\ \mu M$. This type of error arising through experiment flaws is still frequently encountered in the literature. Furthermore, it is not always easy to reach sufficiently high ligand concentrations (due to low solubility) or, conversely, sufficiently low concentrations (due to inadequate sensitivity). It is therefore essential to bear in mind that in some experiments the presence of certain sites may not be detectable and this must be taken into account during their interpretation.

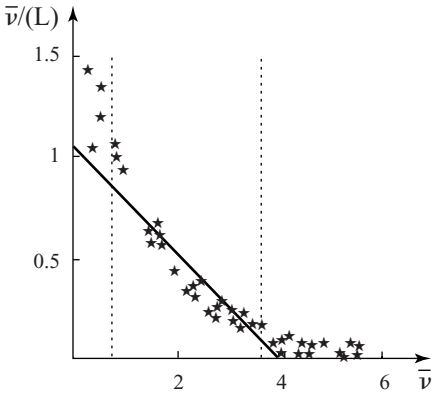


Fig. 2.14 Binding of carbamylphosphate to aspartate transcarbamylase
Dashed lines mark a narrower ligand concentration range.
(From *J. Biol. Chem.*, **251**, SUTER P. & ROSENBUSH J.P., 5986. © (1976) with permission from The American Society for Biochemistry and Molecular Biology)

Amongst the errors found in the literature, it is useful to point out the erroneous interpretations of the non-linear SCATCHARD plots mentioned by NØRBY et al. (1980). *Figure 2.15 illustrates this type of error*. Figure 2.15a represents the false interpretation, which involves comparing the slope of the linear parts of the diagram to the dissociation constant and simply extrapolating in order to have the number of sites of each category. Figure 2.15b represents the rigorous deconvolution of this diagram according to the procedure previously indicated (see Sect. 2.3.3).

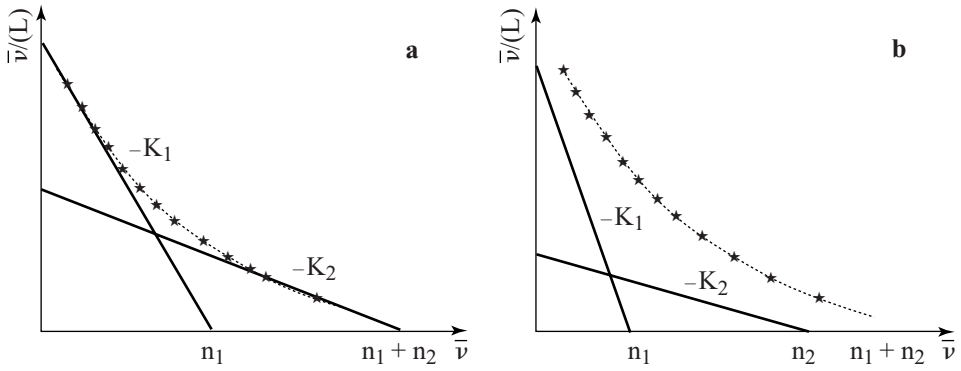


Fig. 2.15 Theoretical SCATCHARD diagrams for the binding of a ligand to two categories of different sites
*(**a**) incorrect resolution of the data – (**b**) correct resolution of the data*
(Reprinted from *Anal. Biochem.*, 102, NØRBY J.G. *et al.*, Scatchard plot: common misinterpretation of binding experiments, 318. © (1980) with permission from Elsevier)

transformed into carbohydrates and oxygen, and then into other nutrient molecules. The biotopes that develop around hydrothermal sources from volcanic faults at the bottom of the ocean are an exception. The life of organisms that constitute these biotopes rests entirely on the capacity of autochemolithotropic bacteria to extract energy from chemical reactions, such as sulphurous oxidation, in order to synthesise organic molecules.

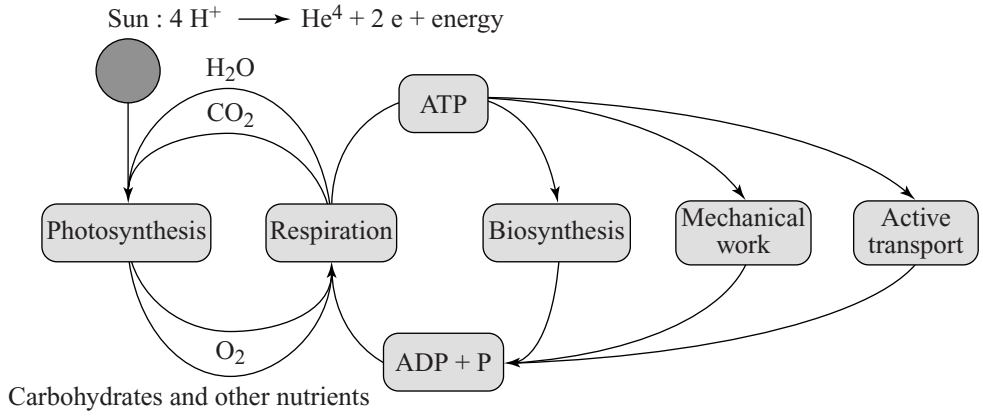


Fig. 3.3 General cycle of matter and energy between the mineral, vegetable and animal worlds

Visible light is a form of radiation or electromagnetic energy. At the extreme temperature found in the sun (6 000 K), a fraction of the enormous quantity of trapped energy within the nuclei of hydrogen atoms is liberated while being transformed by nuclear fusion into helium atoms and electrons:

$$4H \longrightarrow He^4 + 2e + h\nu$$

During this reaction, a quantum of energy is emitted in the form of radiation.

After a series of transformations during which the radiation is absorbed by electrons and atoms, much of the radiation is re-emitted in the form of photons, or quanta of light energy. Therefore, the nuclear fusion reactions in the sun are at the origin of all biological energy on our planet. Also, fuel used by man e.g. coal and petrol, originates from the sun.

During photosynthesis, diverse phenomena occur:

- absorption of radiated energy by chlorophyll,
- conversion of this energy to chemical energy,
- use of chemical energy to reduce CO_2 drawn into the atmosphere and to synthesise glucose. Photosynthesis corresponds broadly to the reaction:

$$6CO_2 + 6H_2O + h\nu \longrightarrow C_6H_{12}O_6 + 6O_2$$

The formation of glucose from CO_2 and H_2O requires considerable energy (686 kcal . mol^{-1}) and hence is a very strongly endergonic process. Thus, a large

9 – TOPOLOGY OF THE ACTIVE CENTRE OF ENZYMES

For most enzymes, there is a disproportion between the size of the enzymatic molecule and the size of the substrate. Such an observation brought about very early the notion of the *active centre*, the fact that a very small proportion of the surface of the enzyme enters into contact with the substrate. However, the concept of the active centre remained for a long time rather poorly defined. For KOSHLAND, the active centre was constituted by all the enzyme atoms that are in contact fixed in the boundaries of the VAN DER WAALS radius with the substrate atoms, meaning those that stay at a minimal distance such that the electron clouds are not perturbed. KOSHLAND distinguishes thus the contact residues and the auxiliary residues, the latter being able to play a role in enzymatic activity (Fig. 9.1).

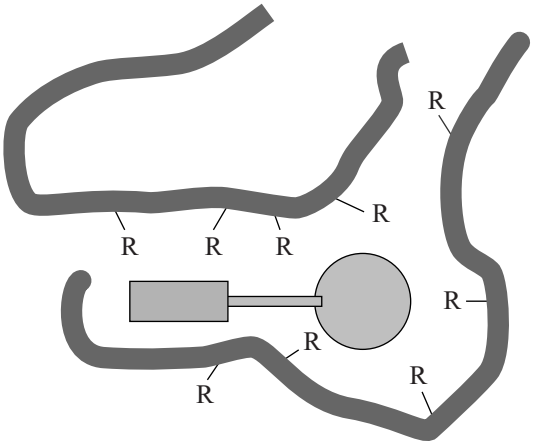


Fig. 9.1 Schematic representation of the active centre of an enzyme with the different residues R that interact with the substrate

Meanwhile, such an idea remains insufficient. It is purely spatial and does not take into account the functional aspect. Indeed, the residues of the enzyme that come into contact with the substrate can have very diverse roles, either interfering with the binding of the substrate or participating directly in the catalysis. Some do not play any role; they are present at the active centre by consequence of the polypeptide sequence and have direct influence neither on the enzyme-substrate association nor on the catalysis. Their modification or replacement by other amino acids in crossing from one species to another, for example, or replacement by site-directed mutagenesis, does not change their activity. On the contrary, certain amino acids which are not bound by VAN DER WAALS forces to the substrate can play an essen-

J. Yon-Kahn, G. Hervé, *Molecular and Cellular Enzymology*,
DOI 10.1007/978-3-642-01228-0_10, © Springer-Verlag Berlin Heidelberg 2010

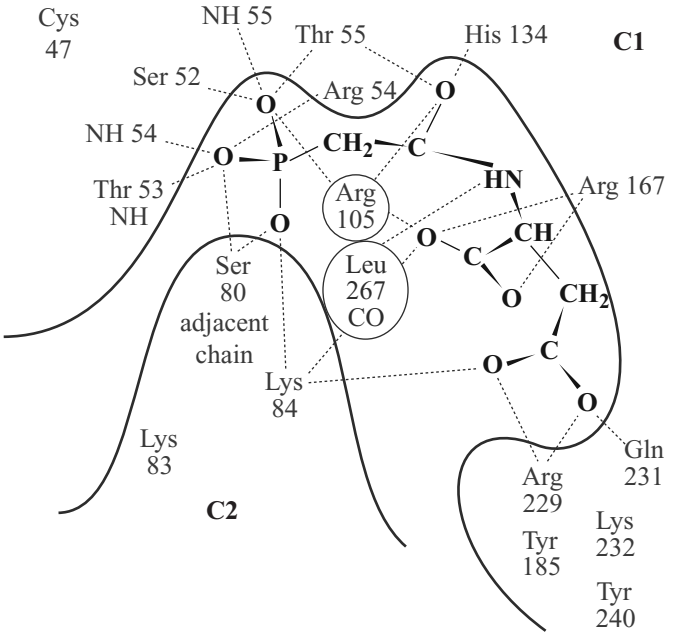


Fig. 10.5 Side chains implicated in the association of N-phosphonacetyl-L-aspartate (PALA) with aspartate transcarbamylase

The dotted lines represent the hydrogen bonds, salt bridges and other polar contacts at a distance of less than 3.5 Å. (Reprinted from *Biochem. Biophys. Res. Commun.*, **136**, VOLTZ K.W. et al., The binding of N-(phosphonacetyl)-L-aspartate to aspartate carbamoyltransferase of *Escherichia coli*, 822. © (1986) with permission from Elsevier)

10.2. ENERGETICS OF ENZYME-SUBSTRATE ASSOCIATIONS

When the dissociation constant of the enzyme-substrate complex K_s is known, it is possible to evaluate the thermodynamic constants that correspond to the formation of this complex. The free energy of complex formation is reliant on the constant K_s by the equation:

$$\Delta G = -RT \ln 1/K_s = RT \ln K_s$$

The values of ΔG obtained for the formation of the MICHAELIS complex are generally weak and always negative; *the formation of the first enzyme-substrate complex is accompanied by a decrease in free energy of the system, it is an exergonic process and therefore spontaneous.*

The study in variations in K_s as a function of temperature permits the evaluation of the variation in enthalpy, ΔH, corresponding to the formation of the complex, according to the equation:

The expression of isotope effects with tritium is given by an analogous relationship. It follows that the comparison of isotope effects of deuterium and tritium are expressed by the relationship:

$$\frac{[(k_{cat} / K_m)_H / (k_{cat} / K_m)_D] - 1}{[(k_{cat} / K_m)_H / (k_{cat} / K_m)_T] - 1} = \frac{(k_{2H} / k_{2D}) - 1}{(k_{2H} / k_{2T}) - 1}$$

Thus the isotope effects of deuterium and tritium that can be determined experimentally will concern only the constant k_2. If no isotope effect is observed, this will signify that k_2 is not the limiting step of the reaction.

It is convenient to distinguish the isotope effects implicating non-exchangeable hydrogen atoms from those rapidly exchangeable with the solvent. For experiments in D_2O, it is important also to know the pH dependence of the isotope effect on the kinetic parameters of the reaction. In enzymes, conformational effects are sometimes observed, in particular modifications of hydrogen bonds in the presence of D_2O which are difficult to encompass.

In Chap. 12, examples will be given concerning particular enzyme systems.

11.3. PRINCIPAL TYPES OF REACTIONS CATALYSED BY ENZYMES

The chemical reactions catalysed by enzymes can be classified into four main categories:

- group transfer reactions,
- oxydoreduction reactions,
- isomerisations, eliminations and rearrangements,
- and finally, reactions implicating the formation or cleavage of carbon-carbon bonds.

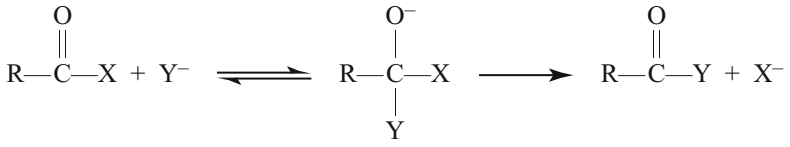
It is important to describe first these diverse reaction types before analysing the characteristics of enzyme catalysis.

11.3.1. GROUP TRANSFER REACTIONS

In this reaction type, an electrophilic part of the substrate, for example an acyl, phosphoryl or glycosyl group, is transferred to an acceptor nucleophile. This latter can be oxygen of the water molecule; there is then degradative hydrolysis. One such mechanism is implicated in enzyme hydrolysis processes of macromolecules (proteins, DNA and RNA, polysaccharides, lipids), and also in the hydrolysis of smaller molecules like acetylcholine. Conversely, in the cell, other molecules carrying a nucleophilic group such as oxygen, nitrogen or sulfur serve as the acceptor in synthesis processes.

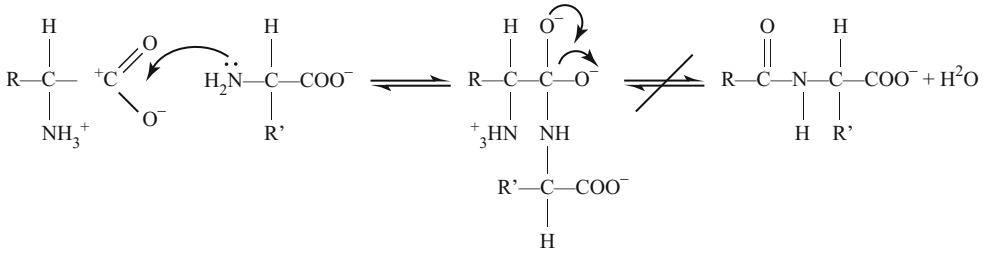
11.3.1.1. ACYL TRANSFER REACTIONS

Reactions of acyl transfer were taken like the previous example to describe mechanisms implicated in nucleophilic catalysis. These are reactions of the following type:



These reactions proceed through the formation of a tetrahedral intermediate as was shown in Sect. 11.1.2.1. When the nucleophile is the oxygen of water, such a mechanism leads to hydrolysis. When the acceptor is an amino acid or a peptide, and the nucleophile is amino nitrogen, there is a transpeptidation.

Protein biosynthesis which implicates the formation of peptide bonds cannot be done by a direct condensation as the following reaction would be:

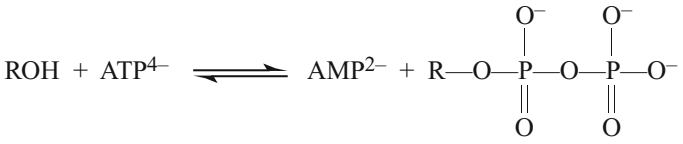


This last reaction can take place only if the carboxylate is activated. For protein biosynthesis in the cellular milieu, the activation of the carboxylate occurs *via* its conversion in phosphoric ester which uses ATP; it is the formation of the aminoacyl adenylate. The phosphate group can be easily eliminated *via* a tetrahedral addition complex. In reality it occurs following transfer reactions of phosphoryl groups.

11.3.1.2. PHOSPHORYL GROUP TRANSFER

The formation of aminoacyl adenylate consists of a nucleophilic attack on the α phosphate of ATP, releasing the pyrophosphate (Fig. 11.14 opposite). The following step is the charge of aminoacyl adenylate on specific tRNA which implies a nucleophilic attack on the carbon of the phosphoric ester bond by the hydroxyl oxygen in position 2′, with liberation of AMP and formation of aminoacyl tRNA. Then a very rapid migration in position 3′ occurs. Finally, these aminoacyl esters form the peptide bond by nucleophilic attack on the carbon of the ester bond by the amino group nitrogen with liberation of the deacylated tRNA.

The attack on the β phosphate of ATP drives a pyrophosphate transfer:



12.2.2.1. STRUCTURAL PROPERTIES

The sequences of more than thirty phosphoglycerate kinases were determined, by either protein sequencing or gene sequencing. They show that this enzyme was particularly well conserved over the course of evolution. The three-dimensional structures of the enzyme of horse muscle (BANKS et al., 1979), yeast (WATSON et al., 1992), *Bacillus stearothermophilus* (DAVIES et al., 1993), pig (HARLOS et al., 1992) and *Trypanosoma brucei* (BERNSTEIN & HOL, 1998) were solved. Outside of some local differences, these structures present great similarities, particularly in the C-terminal part.

Au: Watson et al. (1992), Davies et al. (1993) are not listed in the Bibliography list. Please provide.

The molecule of molecular weight 44 500 is made of a single polypeptide chain folded into two domains of nearly equal size (Fig. 12.22). The elements of secondary structure are organised in repeating αβ motifs; the structure is of the α/β type. Each domain is made of six parallel β segments forming an internal sheet surrounded by helices. In total, 25% of the chain is implicated in the β structures and 42% in α helices. The twelve last residues of the polypeptide chain are strongly associated to the N-terminal domain. Residues 186–189 which join helix 7 and the segment βF (helix V and βG in the yeast enzyme) form the link between the two domains. The C-terminal domain comprises the ROSSMANN fold, characteristic of the NAD^+ binding domain of dehydrogenases. There is no sequence similarity between the two domains which differ also in the detail of their three-dimensional structure. The free enzyme presents a large groove between the two domains, an open structure in the absence of substrates, as was described for other kinases, in particular hexokinase.

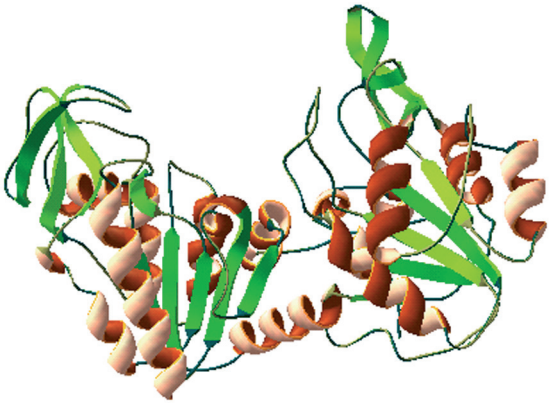


Fig. 12.22 Schematic representation of the phosphoglycerate kinase structure (PDB: 2PGK) showing the two structural domains

12.2.2.2. THE BINDING SITE OF NUCLEOTIDE SUBSTRATES

Radiocrystallographic studies were carried out on complexes formed by the enzyme with either Mg-ADP or MgATP. They reveal only a single binding site for nucleotide substrates; this site, located in the C-terminal domain, is identical for Mg-ATP and Mg-ADP. The enzymes of horse, yeast and *Bacillus stearothermophilus* show analogous characteristics. Figure 12.23 below illustrates the interactions of the horse enzyme with Mg-ATP. Bound to the enzyme, Mg-ATP like Mg-ADP, has a

It is also included in the thermodynamical considerations of WYMAN (1965) on the importance of binding potentials. The concept of coupling, stated by NOBLE (1967), was extensively developed by WEBER, who conceived a protein behaviour analysis method for proteins displaying cooperative effects, in terms of variations in free energy, associated with both ligand binding and interactions between protein subunits.

Au: Wyman (1965); Noble (1967), is not listed in the Bibliography list. Please provide

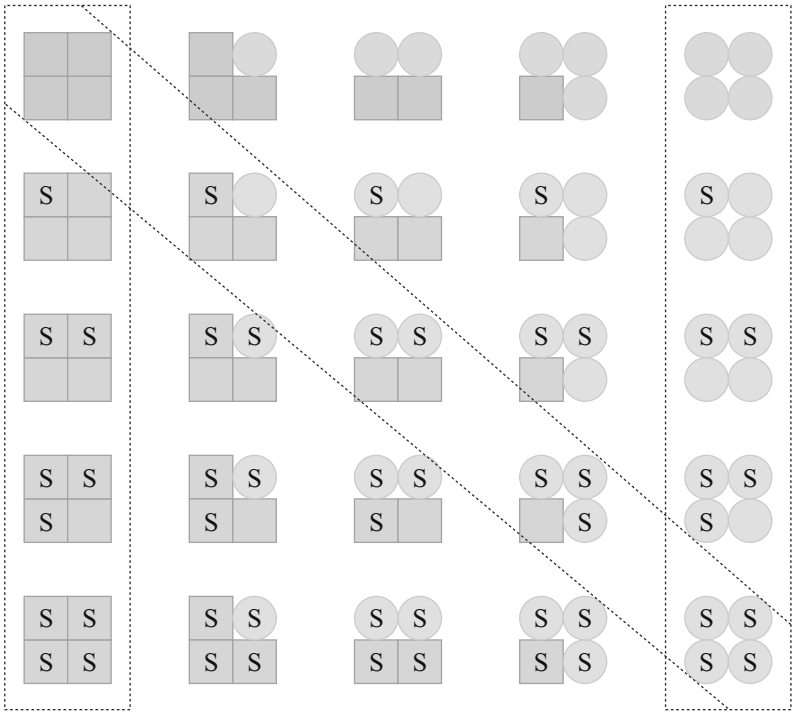
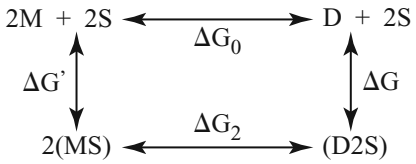


Fig. 13.11 The generalised ADAIR-WEBER model

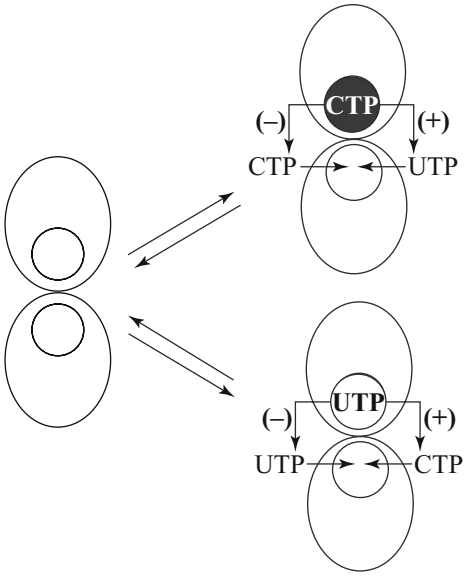
Let us consider the extreme case of a monomer-dimer equilibrium. If the substrate S has a higher affinity for the dimeric form D than for the monomeric form M, it will shift the equilibrium towards the dimer. Every physico-chemical parameter which favours the dissociation will however disfavour the binding of S. The described situation is characterised by the following cycle:



From a thermodynamical point of view, the different values of ΔG correspond to the variations in free energy, which accompany each reaction. The variation in free energy to go from one state to another is independent of the chosen path, so the sum of the variations around the cycle is zero, and:

$$\Delta G - \Delta G' = \Delta G_2 - \Delta G_0$$

site decreases the affinity of the second site for CTP, but increases its affinity for UTP and conversely (ENGLAND & HERVÉ, 1992). The phenomenon is identical if the regulatory subunits (dimers of regulatory chains) are associated or not to the catalytic subunits, showing that in this process there is no interaction between the regulatory subunits in the holoenzyme.



$K_{d\,(UTP)}$ = 810 μm → real $K_{d\,(UTP/CTP)}$ = 10 μM

$K_d1_{\,(CTP)}$ = 9 μm → real $K_d1_{\,(CTP/UTP)}$ = 1.7 μM

$K_d2_{\,(CTP)}$ = 260 μm → real $K_d2_{\,(CTP/UTP)}$ = 70 μM

Fig. 13.31 Model of the interactions between regulatory sites within a regulatory dimer in ATCase
The + and – signs correspond to an increase (cooperativity) and to a decrease (anticooperativity) of affinity, respectively. (Reprinted with permission from *Biochemistry*, **31**, ENGLAND P. & HERVÉ G., Synergistic inhibition of *Escherichia coli* aspartate transcarbamylase by CTP and UTP: binding studies using continuous-flow dialysis, 9725. © (1992) American Chemical Society)

The regulatory signals of ATP, UTP and CTP are transmitted by different paths

Extensive use of site-directed mutagenesis has shown that the transmission of different regulatory signals of the three effectors imply different interface zones between the catalytic chains and the regulatory chains, but also between different domains of the same chain (XI et al., 1991; VAN VLIET et al., 1991; DE STAERCKE et al., 1993). At the interface between the C-terminal region of one regulatory chain and the loop containing the residue 240 of a catalytic chain (R1C4 type interface in Fig. 13.23), two contact zones, close to each other, are specifically involved, one in the transmission of the regulatory CTP signal, the other in the transmission of the regulatory ATP signal.

Au: De Staercke *et al.*, (1993) is not listed in the Bibliography list. Please provide.

The regulatory chain is constituted of two domains, the allosteric domain where the regulatory site is located, and the zinc domain, which is in contact with the catalytic chains. The interface between the two domains is constituted of a hydrophobic pocket in which a tyrosine residue is inserted (Fig. 13.32 opposite). All of the interface is essential for the transmission of the ATP regulatory signal (XI et al., 1994).

In addition, the catalytic domain interacts with the procolipase by the intermediate of the lid, creating a long continuous hydrophobic plateau of more than 50 Å in length. Such a surface is capable of strongly interacting with the lipid-water interfaces covered by biliary salts, preventing their inhibitor effect. Figure 13.52, obtained by modelling, represents the interaction of lipids with the complex.

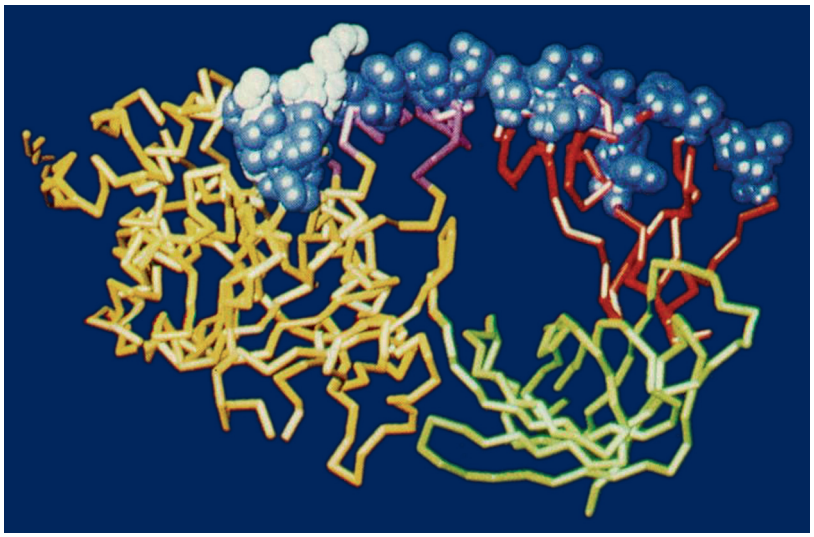


Fig. 13.52 Model of the hypothetical surface of lipid binding (*in white*) to the lipase-procolipase complex

The catalytic domain is in yellow*, the C-terminal domain in green and the colipase* in red*. The hydrophobic residues are represented as blue spheres.* (Reprinted by permission from Macmillan Publishers Ltd: *Nature*, **359**, VAN TILBEURGH H. et al., 159. © (1992))

On the described structural basis, a simplified model of the interfacial activation of the lipase is presented in Fig. 13.53, according to CARRIÈRE et al. (1994).

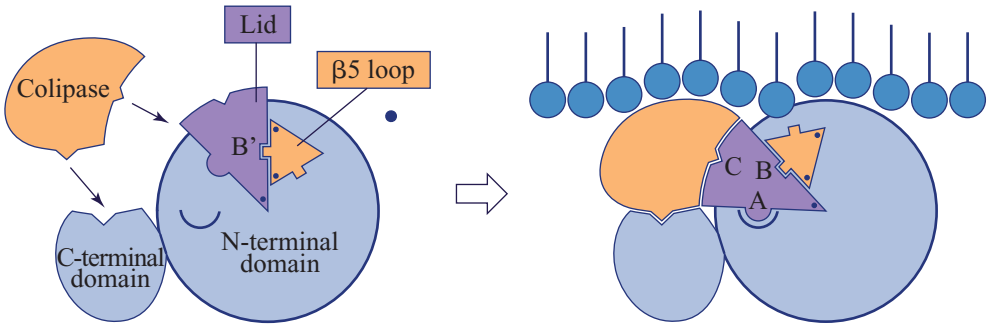


Fig. 13.53 Activation mechanism of the lipase

(Reprinted from *Protein Engineering, Design and Selection*, **7**, CARRIÈRE F. et al., Structure-function relationships in naturally occurring mutants of pancreatic lipase, 563. © (1994) by permission of Oxford University Press)

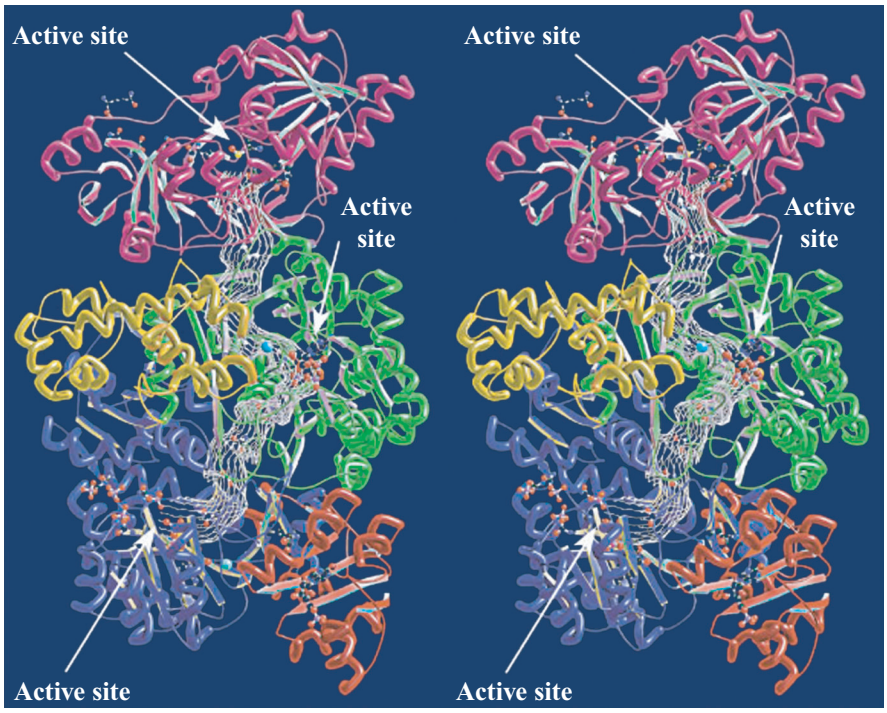


Fig. 15.13 Structure of the αβ heterodimer of CPSase showing the tunnel between the active sites (stereoscopic view)

(Reprinted from *Curr. Op. Struct. Biol.*, **8**, HOLDEN H.M. et al., 679. © (1998) with permission from Elsevier)

15.5. THE PYRUVATE DEHYDROGENASE COMPLEX

At the junction between glycolysis and the citric cycle, the multi-enzymatic complex of pyruvate dehydrogenase catalyses the transformation of pyruvate into acetyl-CoA following the global reaction:

$$\text{pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH}$$

It is constituted by the assembly of multiple copies of three different kinds of enzymes, the component E1 that is a pyruvate decarboxylase, the component E2 that is a dihydrolipoamide acetyl transferase and the component E3 that is a dihydrolipoamide dehydrogenase.

15.5.1. FUNCTIONAL PROPERTIES

The component E1, which has the pyruvate decarboxylase activity, functions in the presence of a coenzyme, thiamine pyrophosphate (TPP) and catalyses the reaction:

$$\text{pyruvate} + \text{TPP} \longrightarrow \text{hydroxyathyl-TPP} + \text{CO}_2$$